

We claim:

1. A method for identifying a thermostable polymerase having altered fidelity, comprising generating a random population of polymerase mutants by mutating at  
5 least one amino acid residue of a thermostable polymerase and screening said population for one or more active polymerase mutants by genetic selection.
2. The method of claim 1, wherein two or more  
10 amino acid residues of said thermostable polymerase are mutated.
3. The method of claim 1, further comprising determining a fidelity of said active polymerase mutant.
4. The method of claim 1, wherein said  
15 mutated amino acid residue is adjacent to an immutable or nearly immutable residue.
5. The method of claim 4, wherein said mutated amino acid residue is immediately adjacent to an immutable or nearly immutable residue.
6. The method of claim 1, wherein said  
20 mutated amino acid residue is in an O-helix of a thermostable polymerase.
7. The method of claim 4, wherein said mutated amino acid residue is adjacent to an amino acid residue corresponding to Arg659, Lys663, Phe667 or Tyr671  
25 in *Taq* DNA polymerase.
8. The method of claim 7, wherein said thermostable polymerase is *Taq* DNA polymerase.

9. A method for identifying a thermostable polymerase having altered fidelity, comprising generating a random population of polymerase mutants by mutating at least one amino acid residue in an active site O-helix of a thermostable polymerase and screening said population for one or more active polymerase mutants.

10. The method of claim 9, wherein two or more amino acid residues of said thermostable polymerase is mutated.

11. The method of claim 9, further comprising determining a fidelity of said active polymerase mutant.

12. The method of claim 9, wherein said mutated amino acid residue is adjacent to an immutable or nearly immutable residue.

13. The method of claim 12, wherein said mutated amino acid residue is immediately adjacent to an immutable or nearly immutable residue.

14. The method of claim 12, wherein said one or more amino acid residues is adjacent to an amino acid residue corresponding to Arg659, Lys663, Phe667 or Tyr671 in *Taq* DNA polymerase.

15. The method of claim 14, wherein said thermostable polymerase is *Taq* DNA polymerase.

16. An isolated thermostable polymerase mutant having altered fidelity, wherein said mutant comprises one or more mutated amino acid residues in the active site O-helix of a thermostable polymerase.

17. The polymerase mutant of claim 16, wherein said polymerase is *Taq* DNA polymerase.

18. The polymerase mutant of claim 16, wherein said mutated amino acid residue is adjacent to an immutable or nearly immutable residue.

19. The polymerase mutant of claim 18, wherein said mutated amino acid residue is immediately adjacent to an immutable or nearly immutable residue.

20. The polymerase mutant of claim 18, wherein said mutated amino acid residue is adjacent to an amino acid residue corresponding to Arg659, Lys663, Phe667 or Tyr671 in *Taq* DNA polymerase.

21. The polymerase mutant of claim 20, wherein said polymerase is *Taq* DNA polymerase.

22. The polymerase mutant of claim 17, wherein said polymerase mutant is a high fidelity mutant.

23. The polymerase mutant of claim 22, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser; Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

24. The polymerase mutant of claim 17, wherein said polymerase mutant is a low fidelity mutant.

25. The polymerase mutant of claim 24, wherein said polymerase mutant comprises substitution of one or more amino acids selected from the group consisting of Ala661, Thr664, Asn666 and Leu670.

5           26. The polymerase mutant of claim 25, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; Thr664Arg; Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and  
10 Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and Asn666Ile.

27. An isolated nucleic acid molecule encoding  
15 a polymerase mutant having high fidelity, comprising a nucleotide sequence encoding substantially an amino acid sequence of Taq DNA polymerase I comprising one or more amino acid substitutions selected from the group consisting of Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu;  
20 Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser; Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

28. An isolated nucleic acid molecule encoding  
25 a polymerase mutant having low fidelity, comprising a nucleotide sequence encoding substantially an amino acid sequence of Taq DNA polymerase I comprising substitution of one or more amino acids selected from the group consisting of Ala661, Thr664, Asn666 and Leu670.

29. The nucleic acid molecule of claim 28, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; 5 Thr664Arg; Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and Asn666Ile.

10 30. A method for identifying one or more mutations in a gene, comprising amplifying said gene using a high fidelity polymerase mutant under conditions which allow polymerase chain reaction amplification.

15 31. A method for identifying one or more mutations in a gene, comprising amplifying said gene using the high fidelity polymerase mutant of claim 22 under conditions which allow polymerase chain reaction amplification.

20 32. The method of claim 30, wherein said gene is amplified by exposing the strands of said gene to repeated cycles of denaturing, annealing and elongation to produce an amplified product.

25 33. The method of claim 32, further comprising determining the presence or absence of one or more mutations in the sequence of said gene.

30 34. The method of claim 30, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser;

Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

35. A method for accurately copying repetitive  
5 nucleotide sequences, comprising amplifying said repetitive nucleotide sequence using a high fidelity polymerase mutant.

36. The method of claim 35, wherein said repetitive nucleotide sequence is in a gene.

10 37. The method of claim 35, wherein said repetitive nucleotide sequence is in a microsatellite between genes.

38. A method for accurately copying repetitive nucleotide sequences, comprising amplifying said  
15 repetitive nucleotide sequence using said high fidelity polymerase mutant of claim 22.

39. A method for determining an inherited mutation, comprising amplifying a gene using a high fidelity polymerase mutant.

20 40. A method for diagnosing a genetic disease, comprising correlating the inherited mutation determined in claim 39 with said genetic disease.

41. A method for diagnosing a genetic disease, comprising amplifying a gene using a high fidelity  
25 polymerase mutant.

42. A method for diagnosing a genetic disease, comprising amplifying a gene using said high fidelity polymerase mutant of claim 22.

43. The method of claim 41, wherein said  
5 genetic disease comprises mutations in microsatellite or repetitive DNA.

44. The method of claim 43, wherein said genetic disease is cancer.

45. A method for determining the prognosis of  
10 a genetic disease, comprising amplifying said gene in claim 41.

46. The method of claim 41, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of  
15 Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser; Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

20 47. A method for randomly mutagenizing a gene, comprising amplifying said gene using a low fidelity polymerase mutant.

48. A method for randomly mutagenizing a gene, comprising amplifying said gene using said low fidelity  
25 polymerase mutant of claim 24.

49. The method of claim 48, wherein said polymerase mutant comprises substitution of one or more amino acid residues selected from the group consisting of Ala661, Thr664, Asn666 and Leu670.

- 5                    50. The method of claim 49, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; Thr664Arg; Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and  
10 Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and Asn666Ile.

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